

Extrapolation of Carcinogenesis Data

by Wil Lepkowski*

Introduction

This is an outline of the state of the art of biomedical research as used to estimate the potential public health consequences of long-term human exposure to chemical agents in the environment. It is a report on the ideas and research findings discussed at a conference held March 10-12, 1976, at Pinehurst, N. C., sponsored by the National Institute of Environmental Health Sciences.

Current methods of estimating the human risk from chronic exposure to low dose levels of chemical agents in the environment rely mainly on data from experiments with laboratory animals. In these experiments, animals are typically exposed to doses low enough to minimize early deaths from acute toxicity and yet high enough to provide assurance of detecting chronic effects, such as carcinogenicity. Since the number of animals which can be employed in testing any one chemical is limited, it is usually necessary to expose the animals to dose levels far above the anticipated range of human exposure in order to be reasonably sure of detecting chronic effects.

The problem of using data on high-dose effects of chemicals in animals to predict the low-dose effects of these chemicals in humans is commonly known as the low-dose extrapolation problem. This includes not only the complex biological problem of inferring human response from data on animal response, but also the biological and statistical problem of predicting effects in large populations exposed to low dose levels from data on small populations exposed to high dose levels.

The metabolic reactions which occur when a given chemical is administered to an animal depend on a number of factors, including among others the species, the specific genetic strain, the sex, and the route by which the chemical is administered. The same chemical can give rise to different metabolites in different animals. Since the underlying

mechanisms through which chemicals can induce chronic diseases such as cancer are not completely understood, it is currently not possible to relate these metabolic differences in a quantitative way to differences among species in the sensitivity to a given chemical. However, experimental results in laboratory animals are a guide to probable results in humans.

Extrapolation from high dose levels to low dose levels is also strongly affected by incompleteness in current knowledge of chronic disease mechanisms. Several biologically plausible statistical models seem to fit the dose-response data more or less equally well in the high-dose range and yet yield markedly different estimates of risk at low dose levels such as humans might encounter. These differences in predictions are caused by different assumptions about the relation between dose and response. The exact nature of the relationship between dose and response is not known and is probably different for different classes of chemicals.

Although the scientific groundwork necessary for a fundamental understanding of chronic toxicity is incomplete, study of the practical regulatory problems cannot be deferred until all of the basic research has been completed. Thousands of new chemicals come into use each year, and the public health hazards associated with both the new and the existing chemicals need to be evaluated as quickly and as comprehensively as possible.

The Pinehurst conference was an attempt to bring together scientists engaged in basic and applied biomedical research on the health effects of chemicals in the environment, regulatory staff members, and representatives of industry, public interest groups, and the scientific press.

Early and Quick Indicators of Carcinogenicity: Presumptive Tests

The first step in "extrapolation" begins with determining whether or not a substance is car-

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cinogenic. The hope is that relatively rapid tests will become available for determining carcinogenicity and mutagenicity. These tests, involving mainly single cells organisms and mammalian cells, come under the heading "presumptive," and Gary Flamm of NCI summarized the state of the art in that field of testing. Three categories of presumptive tests were outlined that are useful as predictors of carcinogenesis or mutagenesis. The first assesses the potential of a substance and/or its metabolites to damage and/or interact with DNA. The second category is the mutational assay system in cultures of single cells, such as bacteria, other microbial organisms, and mammalian cells. Here, mutational changes are simply observed as indicators of the carcinogenicity of the chemical. There are two major classes to this category of test: those in which the cell responds to highly specific changes in its DNA, and those which respond to any type of cellular damage. Flamm reported that 85–90% of known carcinogens are positive in the more promising single-cell tests system, the Ames test. But he also reported that about 10% which show up as positive have not been shown to be carcinogenic in animal tests.

Said Flamm: "The extent of correlation depends on what classes of chemical carcinogens we are looking at. Chances are that if we looked at just estrogens, we might find absolutely zero correlation, whereas if we were looking at nitrosamines the correlation might be 100%."

In other words, these tests can only be expected to detect carcinogens which act directly on cellular DNA. Indirect carcinogens, which induce cancer by other mechanisms, may have little or no mutagenic potency.

The final category reviewed by Flamm was neoplastic transformation, which measures the development of neoplasms in normal cells grown in tissue culture. Such cells when transplanted into the appropriate host animals show all properties of cancer cells. The problem, he said, is that these cells are difficult to grow in tissue culture.

"Presumptive tests," he said, "will eventually prove useful in providing additional evidence for deciding upon marginal data on the carcinogenicity or mutagenicity of a chemical derived from animal experiments and in studying the interaction between two or more substances. Additionally, they should prove useful as research tools for investigating the molecular targets of carcinogenicity, and in determining what specific events are obligatory to the process of carcinogenesis. As knowledge accumulates, the presumptive tests will provide useful information in the extrapolation of data from species to species and from high dose to low."

But as S. Weinhouse of Temple University said in his summary of the conference, "presumptive tests are not yet ready to replace the costly, time-consuming, uncertain assays in animals."

Reporting on the status of DNA repair as a presumptive indicator of resistance and susceptibility to carcinogenesis, Ann Mitchell of Stanford Research Institute concluded that many questions need answering before the DNA repair approach can be used as a viable tool. "In particular," she said, "more extensive investigations are needed before we can determine with confidence individual variability, tissue specificity, or the potential hazards of the large number of as yet untested chemical agents."

Where does this leave the DNA repair approach as a technique regulators can look to? Mostly as a prescreen for identifying potentially hazardous agents. Studies are underway to explore the possibilities of using DNA repair to correlate *in vitro* and *in vivo* exposure, to correlate measurements of DNA repair in animal systems with those in human cells, to determine tissue susceptibility to various hazardous agents, to monitor the effects of human exposure to hazardous agents, and to predict the individual variation in response to exposures. She said an important potential lay in identifying high risk subpopulations.

A vast amount of research still needs to be done on the methodology itself before DNA repair can contribute much to decision making at the policy level. Adding to the methodological problem is the enormous diversity among animals and humans in the quality and level of their repair mechanisms. Some systems repair more easily than others. Some, through normal genetic variability, are deficient in specific repair enzymes.

Animal-to-Man Extrapolation: Report of Meselson Panel

Matthew Meselson of Harvard University was to have reported to the conference on the animal-to-man extrapolation study done by a National Academy of Sciences subcommittee he headed as part of a larger study on pest control practices and prospects. He was unable to attend, and instead Marvin Schneiderman of the National Cancer Institute substituted with a highly abbreviated summary of Meselson's findings.

Meselson's panel phrased its major conclusion this way: "Although there are major uncertainties in extrapolating the results of animal tests to man, this is usually the only available method for quantitative risk estimation. Despite the uncertainties,

enough is known to indicate what dependencies on dose and time may operate and to provide rough predictions of induced cancer rates in human populations."

Meselson's conclusion, said Schneiderman, was that the best relationship between animal dose data and human dose was a direct milligram per kilogram relationship from the most sensitive animal species to man. But there are some chemicals for which man appears to be a very much less sensitive animal than the most sensitive mammalian species. Yet, the opposite can hold true for other chemicals. Meselson thus raises the question of whether there are some factors to use in establishing human safety out of animal data.

Metabolic Variability among Species

Richard Adamson of the National Cancer Institute provided a detailed overview of species-to-species variation. There are the well acknowledged internal and external variations in the response susceptibility of animals to toxic agents, as well as such administration factors as route, volume, particle size, vehicle composition, and dosage. But even if all administration factors are held constant, species still vary in response to foreign compounds—such as differences in the way the animal absorbs, distributes, metabolizes, and excretes the compound.

He further reviewed the anatomical and physiological differences, such as the absence of the gall bladder in the rat, also the presence or absence of viruses in the host animal. ("This is something we tend to forget," he said. "We have inbred C-type particles into the genome." And perhaps it's the interaction between these compounds and the virus, he indicated that may cause the cancer in the animal. What can one believe from animal studies if that is the case?)

The immune state of the animal, diet, and DNA repair mechanism differences all come into play. He presented data too showing the vast differences in the rate of penetration of organophosphorus compounds through the excised skin of various species. It differs about 30-fold among the pig, rabbit, and rat.

Adamson's message was the need for better care in experimentation and observation. His implication was that the human species is probably stronger constitutionally than lower species and probably more resistant to cancer. He said that the incidence of liver cancer seems to have been dropping despite the increase of chemicals in the environment. But it was also pointed out in commentary

that other cancers are in fact on the increase, DDT or not.

There was a swirl of other commentary on the dubious advisability of "swamping" test animals with high dosages of carcinogens. The modes of action may be different at high dosages than at low and can thus obscure not only the mechanism of carcinogenesis but also introduce factors that may be irrelevant to the natural conditions, where dosages are usually low.

Further Differences in Metabolism Within Species, Within the Same Compound

There is distinct species differentiation in the way animals handle metabolically different dosages of the same and different compounds. R. T. Williams of St. Mary's Hospital Medical School, London, gave an extensive review of such differences. Studies with such reference compounds as phenylacetic acid, sulfadimethoxine, quinic acid, coumarin, 4-hydroxy-3,5-diiodobenzoate, and methanol have shown over the years that Old World monkeys and occasionally New World monkeys are similar to man in metabolic events that lead to cancer. But the more species one studies, the more complicated the picture tends to become.

With five arylacetic acids—phenyl-, *p*-chlorophenyl-, indol-3-yl-, and 1-naphthyl—man and the rhesus monkey are similar. But the pattern of conjugation depends on the arylacetic acid, since with phenyl-, *p*-chlorophenyl-, and indolyl acetic acids, the main conjugate in both species is the expected glutamine conjugate. But with 1-naphthylacetic acid, the glucuronic acid conjugate is formed, and with *p*-nitrophenylacetic acid, no conjugate at all is formed in either species.

It is indeed complex, and the metabolic taxonomy grows and grows. High dose and low dose metabolism differs significantly too. "In general," he said, "it seems probable that the extent of metabolic pathways will change with increase in dose, since it is to be expected that different mechanisms have different capacities. And furthermore, some compounds can induce their own metabolism and possibly one pathway more than another."

Significance of Time in Extrapolation

Daniel Zaharko of the National Cancer Institute introduced a concept that he said was usually over-

looked in toxicity studies that extrapolate animal data to man. It is not sufficient, he said, simply to extrapolate animal concentration levels for a reasonably accurate assessment of the drug's behavior in man. Time is an equally important variable. By time he meant the rate at which the chemical is admitted and metabolized. These rates differ drastically from species to species, as obviously does metabolism itself.

Thus, the importance of proper scaling from species to species is a frequently neglected factor. "There are scientific reports that continue to criticize toxicity studies in small animals as being not relevant to man because of the much larger doses used in animals," he said. Moreover, there is a lack of appreciation for the higher metabolic rates and higher clearances that generally exist in experimental animals. Other factors affecting delivery to intracellular compartments are blood flow, mixing volume, membrane permeability, and binding.

Zaharko in his research used computer models to simulate such factors and give insight into their relative significance in determining concentration and exposure time at the actual site of action.

If concentrations at the active site can be predicted and if biochemical mechanism is known, then ways of counteracting the effect may be developed. When environmental exposure is inevitable, use of another substance to protect against toxicity could be effective.

Diversity in Susceptibility to Cancer in Human Beings

Louise Strong of the University of Texas School of Medicine reviewed the wide range of susceptible subgroups within the human population to various cancers and various carcinogens. Chromosomal instability, genetic variability, and different types of physiological disorders all influence environmental susceptibility. Too, environmental agents as well as radiation could induce an existing predisposition to activation. "Any factor, endogenous or exogenous," she said, "including growth stimulating factors, hormones, drugs, depressed immune system, etc., which might give the mutant cell an advantage may increase the probability of malignancy."

Study of subgroups susceptible to carcinogenesis, she indicated, and definition of the mechanism of their susceptibility is critical to any attempt to extrapolate data from animal to man or from high dose to low dose response. "Even if carcinogenesis can be reduced to a simple two-step mutational model, man is not homogenous with respect to mutation

rates, elimination of mutant cells, growth control, metabolism of potential mutagens, or the number of mutational steps necessary for malignancy," she said. "Study of each uniquely susceptible subgroup may contribute to our understanding of carcinogenesis in general, and may provide complementary *in vitro* systems for the evaluation of potential mutagens."

Strong's paper further increases the sense of complexity that permeates the field of research leading to standards development. She pointed out in considerable detail the many variations of susceptibility—so diverse as to be virtually beyond the limits of public health authorities to fully protect everyone in any given community. With so many persons susceptible in so many potential ways, the "average person" protected by a consensus standard becomes unknowable.

Can the Complexities be Simplified?

While Strong presented an inventory of the many faces of variability in susceptibility, W. W. Weber of the University of Michigan used one class of chemicals, the aromatic amines, to illustrate further the problems of extrapolating carcinogenic data from animals to man. "There is new knowledge derived from biochemistry, molecular biology, microbial and human genetics, and pharmacology, and from various levels of biological organization that must be organized from predicting risks to man," he said.

No one species can serve as the model for metabolism of all compounds in humans. Many reactions are involved in the transformation of an environmental chemical to a carcinogen. For example, aminofluorene becomes acetylaminofluorene (AAF), and AAF is probably not the ultimate carcinogen, whatever the species. Very little, Weber said, has been reported on the range of variability in deacetylation in the metabolism of carcinogens.

It may be possible at some future time to determine human susceptibility to environmental carcinogens, he pointed out. The human population may be divided genetically into rapid acetylators and slow acetylators, using isoniazid as a tracer compound. "It seems," he mused, "that people who are genetically rapid acetylators of isoniazid might also be expected to transform aminofluorene and other hazardous amines to activated carcinogenic forms more rapidly than slow acetylators." Weber and his group are studying the problem.

Not Likely Soon

David Clayson said science simply has not progressed to the point of having sufficient details to make assured species-to-species extrapolations. The latency periods (elapsed time from first exposure to clinical manifestation of the disease) differ between mice and men. These are vast differences, and what is more, they are poorly understood.

Clayson said it was necessary to break the carcinogenic process down to its "constituent parts" before valid extrapolations can be made across species boundaries. "I don't feel nihilistic," he said, "but I feel that research on the activities of the proximate carcinogens needs to be done. The quantification of these factors will entail a great deal of work with each carcinogen in each tissue. This effort has been largely ignored by cancer researchers who have apparently more exciting things to do."

But the problem, he said, is that the work will take many tens of thousands of animals to establish the carcinogenic response down to dose curves at the 1% level. "More theoretical considerations may have to be used to establish acceptable carcinogenic risk levels to a relevant level of one in 10^6 to one in 10^8 because the cost of direct experimental approaches would be prohibitive," he concluded.

Significance of Time in Low Dose Effects: the Latency Period

A major focus of controversy in the debate over environmental carcinogenesis and establishment of standards is whether time, or the latency period will nullify the carcinogenicity of chemicals in small doses. Hardin Jones of the Donner Laboratory of the University of California at Berkeley, discounted concern over cancer from low dose levels by research data showing that the cube root of the dose is inversely proportional to the time-to-tumor. That means, said Jones, that there can be assumed to be "practical thresholds," relating concentration to the time expected before neoplasms form. "If they occur beyond the lifespan," he said, "the cost is nil because a practical threshold of effect has not been exceeded."

Jones drew on data from radiation effects on workers who manufactured watches containing dials made of radium and on the atomic bomb casualty data. Both sets of research, he said, followed the cube-root law.

"If we take seriously the things I said about time," he said, "then the extrapolations of known carcinogens to very low doses give us the peace of

mind of thinking that when we reduce the levels of exposure to below 1/1,000 of the level that will produce measurable experimental results in animals in the latter half of their life span, the theoretical tumors we can calculate on the basis of underlying molecular chain of events will not happen during the animal's lifetime—probably not for five lifetimes."

Jones did concede the need to do considerably more research to provide a verifiable data base for the human exposure to chemicals—not just radiation. "The big problem is that we don't have the money or the resources to resolve these issues," he said.

The notion of "practical thresholds," introduced by Hardin Jones, was criticized by Elizabeth Scott of the Statistics Department, University of California at Berkeley. She said it amounted to equating small risks with zero risks. The fact that the expected time-to-tumor, conditional on not having died from a competing cause of death, may be far in excess of usual animal lifespans just reflects a low probability of developing a tumor before dying of something else. The extrapolation problem involves estimating these probabilities. Similar objections were voiced by Richard Peto of Oxford University. He said his analysis of the same data had led him to conclusions different from those which Hardin Jones had drawn.

Extrapolation: The Mathematical Approaches

Before resuming this general account of the extrapolation conference a small primer on the mathematical approaches is in order. This is a description of some of the mathematical bases for risk extrapolation, taken directly from the report on Contemporary Pest Control Practices and Prospects from the National Academy of Sciences:

Threshold Hypothesis

The threshold hypothesis assumes that there is a dose below which cancer induction cannot occur. An examination of published dose response data for chemical carcinogenesis in laboratory animals provides no clear indication of a threshold for any carcinogen. In a review of 151 dose-response curves, none was found to be clearly inconsistent, in a manner suggesting a threshold, with both the single event and the probit hypotheses discussed below. Neither is there any adequate theory of chemical carcinogenesis that would require the general existence of thresholds. Thus, even if a threshold is postulated, there is presently no empirical or

theoretical basis for determining the dose at which it may occur. Unless and until this can be done, the threshold concept does not provide a practical basis for risk estimation.

Single-Event Hypothesis

The single-event hypothesis assumes that the induced incidence of cancer is directly proportional to the dose, all the way from the lower incidence levels that can be measured in animal experiments of practical size down to zero dose and zero response. In other words, below an induced incidence of about 10%, the dose-response curve is, for practical purposes, a straight line. This would result, for example, if cancer is induced by a single cellular event, the likelihood of which is directly proportional to the dose of carcinogen. An essentially linear dose-response relationship can also result under much more general assumptions, so long as the carcinogen in question simply adds its effects to those of other carcinogens already present.

The single-event hypothesis is in agreement with the limited data available for man. The induction of leukemia by ionizing radiation from nuclear explosions is compatible with a linear dose response down to an induced incidence of about 0.1%, the lowest incidence for which the available data can meaningfully be analyzed. Other data on the induction of various types of cancer following radiation, although less extensive, are likewise compatible with linearity. The dose response relating the incidence of lung cancer in man to the average number of cigarettes smoked per day is also compatible with the single-event hypothesis. In this case, the data can be analyzed down to an induced incidence of approximately 2%.

Animal experiments are not usually conducted on a scale large enough to measure induced incidence below a few percent. For some carcinogens in some investigations, the dose-response relation is compatible with the single-event hypothesis, while in other cases it is not. However, it is quite possible that a dose-response departing from the single-event hypothesis at high induced incidence may nevertheless converge to a linear relation at lower incidence values.

Probit and Other Hypotheses Implying a Dose-Response Curve That Is Concave Upwards

This class of hypotheses assumes that there is no threshold for a population but that the incidence at

doses below the lowest tested is less than that implied by the single event hypothesis. Below a response of a few percent, such a relationship between dose and response is described by a smooth curve that is concave upwards. For example, the incidence of skin tumors produced by surface application of benzo[a]pyrene in the mouse has been found to vary as the square of the amount of chemical applied over the dose range examined.

Another dose-response relation that is concave upwards at low dose levels is the probit curve. It assumes that the sensitivity of individuals in a population to chemical carcinogenesis is a gaussian function of the logarithm of the dose. The probit dose-response curve is S-shaped, with a slope that at first increases and then decreases as the dose is lowered. Its use requires the choice of an adjustable parameter, the probit slope, that describes the narrowness of the presumed gaussian distribution of sensitivity to carcinogenesis in the population at risk.

The probit slope may be estimated from dose-response data at high incidence, as determined in an animal experiment, so long as the experimental data is compatible with a probit curve. For sufficiently low dose levels, the probit extrapolation always predicts a lower incidence than does the single-event hypothesis. However, for a value for the slope that is well in the range of values found for various carcinogens in animal experiments at high incidence, the probit extrapolation for lower doses does not differ by more than a factor of ten from the incidence predicted by the single-event hypothesis down to an induced incidence of about one per 100,000 exposed individuals.

Estimates of Carcinogenic Hazard

Until more is known about the mechanisms of chemical carcinogenesis, any method of extrapolation to predict cancer rates at doses much lower than tested will remain partly a matter of conjecture. However, the single-event hypothesis probably provides an upper limit for induced incidence estimates at low dose levels and is compatible with the very limited human data on carcinogenesis at intermediate response levels. It would therefore seem prudent to employ the single-event hypothesis in making risk estimates, at least for those carcinogens for which the dose-response curve from animal experiments approaches linearity at the lower response levels that can practically be studied. In that case, the estimated total lifetime incidence in man resulting from continuous exposure to an environmental carcinogen would be the lifetime incidence

for continuous exposure to the same total dose per body weight found by extrapolation of the animal data under the single-event assumption. For risk assessment, the resulting estimate would then be subject to adjustment to allow for statistical uncertainty in the input data.

Cancer Age Distribution Is Dose-Independent and Risk Is Dose-Proportional

Richard Peto of Oxford University proposed a pair of statistical dogmata which he said should be the foundation for regulatory action. The first stated that at low dose rates, the age distribution of extra tumors beyond those expected from background causes is independent of dose rate. That is, fewer extra tumors would occur at low dose rates than at high dose rates, but the ratio of the total extra tumors to the extra tumors occurring before age 50, for example, would be the same at low dose rates as at high dose rates. Second, that at low dose rates the expected number of extra tumors is proportional to the dose rate.

"There may be a few exceptions to my conclusions," he said, "but the arguments are very general and so regulatory agencies should, in any particular case, expect this dogma to apply unless they have specific evidence that, in the particular case that interests them, the dogmas are false. Because of the wild, outbred heterogeneity of humanity, even agents with threshold-type action in most individuals will also probably comply with the dogma."

There are two main objections to his dogma, Peto said. The first assumes thresholds exist, while the dogmas say that they effectively do not, because "background" carcinogens are persistent and are increasing at rapid rates. Thus, when toxicologists debate what concentration between zero and 10^{20} molecules is safe, they disregard the fact that 10^{20} molecules may already exist as background.

The second objection, said Peto, states that when high dosages of carcinogens are given to animals, the time T_{50} until half the animals have cancer is roughly proportional to $d^{1/3}$. This has led to the hope," he said, "that at low enough dose levels almost all tumors would occur long after age 100, and so would be irrelevant to the human condition. The point is that doses given in these experiments were so high that each animal would have developed several tumors during its natural lifespan, each with a similar age distribution. While the expected time, or latency period, would increase with decreasing dose, this would not continue indefi-

nately. If the expected number of tumors per animal is only 0.1, for example, then so few animals would get two or more tumors that the effects of the first tumor obscuring later tumors will be negligible.

Because of the statistical uncertainties in the differences between the numbers of tumors found in a control group of animals and a treated group of animals, cancer risks lower than about 10% cannot be accurately characterized, even by large experiments.

Statistical Approaches: Different Models Yield Markedly Different Extrapolations

Charles Brown of the National Cancer Institute looked at the statistical uncertainties associated with low dose risk extrapolations. Uncertainty has two main statistical origins, he reported, sampling variability and the more common uncertain choice of a specific dose-response model. He showed that a wide range of low dose extrapolations, spanning several orders of magnitude, can be considered statistically consistent with a given set of dose-response data from tests in the high dose range. Experimental data on dimethylnitrosamine in female rats were used to illustrate his points.

In this paper, Brown introduced the key point that models which appear to fit the data well in the high dose range can still yield low dose extrapolations differing by several orders of magnitude, despite their agreement at high doses.

"Use of a general multiparameter model is one approach that should give good fits to most experimental data and would lead to variability estimates that include both sampling and model-specific variation," he concluded.

Low-Dose Linearity Cannot Be Ruled Out Statistically, Even By Large-Scale Experiments

Using a multiparameter model of the type mentioned by Brown, Harry Guess of NIEHS and Kenny Crump of Louisiana Tech University analyzed animal data for the chemical carcinogens vinyl chloride, dieldrin, DDT, and dimethylnitrosamine and for ionizing radiation. Their aim was to resolve the controversy between probit and single-event extrapolation by using a model general enough to admit both dose-response curves in which risk is proportional to dose in the low-dose range (as in the single event model) and curves in which risk decreases much more rapidly with de-

creasing dose (as in the probit model). For a given set of data their computer program calculates the dose-response relationship under which the experimental results have the highest probability and also calculates upper confidence limits on risk as a function of the dose.

They found that the "most likely" dose-response relations for these chemical carcinogens as well as for ionizing radiation are linear at low doses. In addition, they used computer simulations to conclude that it appears extremely unlikely that even a large-scale experiment involving several thousand animals per dose could conclusively rule out the possibility that the dose-response curve becomes approximately linear in the range of risk below about one percent.

"And when background is present," they said, "it is all but impossible to reject the hypothesis of near linearity at low doses. For example, by changing the outcomes of only 11 out of 8,000 animals in a set of data it is possible to change the form of the best-estimate dose-response relation from one that is highly nonlinear to one that is approximately linear at low doses. This implies that the upper confidence limits on risk will virtually always be linear at low doses, even though the best estimates may be highly nonlinear."

"Our results have implications which should be considered by anyone who intends to design a large scale experiment to measure the shape of dose-response curves for chemical carcinogens in very low dose ranges. When both very flat curves and gradually sloping curves are considered together, it is extremely difficult on mathematical grounds (even with nearly perfect data) to reject the hypothesis that the dose-response curve is nearly linear in the dose range corresponding to increased risks over background of about 10^{-4} or less."

Verification of Linearity at Low Dose by Radiation Experiments

Charles H. Nauman and Arnold H. Sparrow of Brookhaven National Laboratory described experiments on plant mutations using ionizing radiation and gaseous forms of several suspected chemical mutagens. Their study showed that a dose-response curve for mutational response could be linear at low doses and nonlinear at high doses. These results showed that if only the high dose data had been considered and the dose-response curve had been extrapolated graphically on log-log paper into the low dose range, the mutagenic potential of the radiation would have been underestimated. Results with the chemicals were considerably more scattered.

R. Lowry Dobson of Lawrence Livermore Laboratory presented some early results on the biological effects of tritium at low levels and concluded that the biological effectiveness of this insufficiently studied substance does not change appreciably with dose or dose rate.

He found, on the basis of studies with animal oocytes, that the relative biological effectiveness of tritiated water is higher for low level, protracted exposure than for short, higher levels. He said it also seemed possible to extrapolate from short-exposure to tritiated water to chronic exposure. "The possibility has not been excluded," he said, "that a significant degree of especially effective subcellular microdistribution of tritium atoms may occur with protraction of exposure."

Contributions to Extrapolation from Epidemiology

Observation and measurement of the effects of toxic chemicals on workers exposed to them over several years can be of invaluable help assembling the statistical data needed for reliable extrapolation. P. Enterline of the University of Pittsburgh studied the results of asbestos exposure affecting 17,800 asbestos workers. Again, the results were to cast doubt on the easy assumption that thresholds can be a basis for setting numerical standards.

He not only verified that very little difference exists in the effects between a single year's exposure and continuous exposure; he also showed that there remains significantly increased cancer risks for asbestos exposure below the current occupational health standard. "The current regulatory limit value for asbestos is 5 fibers per cubic centimeter (f/cc) averaged over an 8-hr day, not to exceed 10 f/cc at any one time," he reported. "As is true for most standards, no epidemiological data on factory workers exposed at this level are available. Our model shows that 4 f/cc predicts a 20% increase in respiratory cancer among factory workers after 40 yr and a 40% increase after 60 yr. His model consisted of a series of assumptions based on past data gathered on asbestos toxicology.

Peter Infante, substituting for Joseph Waggoner of the National Institute for Occupational Safety and Health, reviewed a series of data relating epidemiologic studies of vinyl chloride and a number of anesthetics to high incidences of cancer and birth defects. In doing so, he asserted that epidemiologic data have not been given strong enough emphasis in determining the safety of toxic substances.

"The epidemiological approach for mutagenesis or teratogenesis," Infante said, "being the equiva-

lent of short-term *in vitro* or *in vivo* tests, can be a useful test for controlling many carcinogens without waiting for the long latent period so characteristic of occupationally induced cancer."

Thresholds

There are two ways of looking at thresholds for carcinogens: with the scientific fascination of the molecular researcher, and with the view of the regulatory official who needs answers quickly. The scientific facts were reviewed by Hans Falk of NIEHS and the more philosophical facts and policy implications by David Rall, also of NIEHS.

Determination of thresholds are terribly complicated, Falk said, and elaborated. "Thresholds can be moved back and forth by a number of factors that are temporal in nature. In other words we may be having this threshold today and that threshold tomorrow on the basis of either intercurrent disease, dietary splurges, or exposures to other chemicals that may help in this process."

He said consideration of thresholds would have to take into account the fact of deactivating mechanisms that normally occur in the organism. Enzymes such as glutathione transferase deactivate carcinogens. "This is not an imaginary reaction that we can put on any blackboard for the sake of saying what could happen. This does happen."

Falk outlined the highlights of repair mechanisms and further outlined the complexities of the carcinogenic process. "Many of the mutations that may be produced by a chemical may not be terribly significant," he said. "It is not the fate of most molecules to end up as active carcinogens, particularly at minute dose levels."

But those that do, or that might, are the concern, and scientific facts and informed speculation must lead to regulatory action. Scientifically, thresholds are fascinating, said Rall. But as a basis for policy decisions over standards, he had doubts.

Each new chemical that is added to the environment introduces that much more added risk, he said, notwithstanding the fact that some chemicals may neutralize the carcinogenic potential of other chemicals. The main issue, he said, was over the long term effects of today's chemicals.

Carcinogenic effects are irreversible, he said. Even removing a chemical from the environment will not reverse the carcinogenic process once it has been triggered by that chemical. It is perfectly logical, said Rall, to believe that low concentrations of even the most potent carcinogens will not cause cancer. "But to design an experiment to show whether or not such a statement is correct would take enormous resources and, even if the experiment could be performed, the answer would probably be suspect," he said.

The human population is diverse and thresholds should reflect that diversity. There is no one threshold that would cover the public. The issue, then, is not really thresholds, he said, but one of adding new carcinogens to the pool of carcinogens already present in the environment.

"If thresholds do exist and the regulatory decisions are based on a no-threshold concept there will be short-term economic losses," he said. "If thresholds do not exist and the regulatory decisions are based on thresholds, then there will be fewer short-term economic losses. But we would face a future of damaged human somatic and germinal DNA and an increased incidence of neoplastic disease."